MECHANISM FOR CELL SEPARATION BASED ON SIZE AND DEFORMABILITY USING MICROFLUIDIC RATCHETS

S.M. McFaul, B.K. Lin and H. Ma*
University of British Columbia, CANADA

ABSTRACT
We present a mechanism for separating cells based on size and deformability using microfluidic ratchets created using micrometer-scale funnel constrictions. The force required to deform individual cells through such constrictions is directionally asymmetric, enabling rectified transport from oscillatory flow of the bulk fluid. Combining ratcheting with simple filtration enables cell separation based on size and deformability. Based on this concept, we developed a microfluidic device using a 2D matrix of funnel constrictions. We demonstrate highly selective separation of two cell types while retaining viability, study the effect of oscillation flow pressure, and confirm the irreversible nature of the ratcheting process.

KEYWORDS: Cell Sorting, Cell Separation, Deformability, Ratchet, Filtration, Microfluidics

INTRODUCTION
Filtration using an array of microscale constrictions has long been considered as a technique for separating cells based on their physical characteristics [1-3]. The selectivity of these techniques is limited by clogging whereby cells trapped in the filter alter its hydrodynamic resistance unpredictably. Reversing the flow to unclog the filter reverses the separation because of the low-Reynolds number flow in these systems. We present a mechanism for cell separation based on their mechanical properties using oscillatory flow through a microfluidic ratchet. This ratchet is formed using tapered funnel constrictions, where the end of a taper is smaller than the diameter of a single cell (Figure 1). We recently showed that the pressure required to deform single cells through such a constriction along the direction of the taper is less than the pressure required against the taper. We further showed that precisely controlled oscillatory flow of the bulk liquid could enable unidirectional transport of the deformed cells [4]. Combining the ratchet transport of smaller and more deformable cells with the filtration of larger and less deformable cells enables separation based on a combination of size and deformability, or more precisely, squeezability. The ability to separate cells using an oscillatory flow improves selectivity by preventing clogging, and allows for extraction of the cells after separation.

DESIGN
We implemented this cell separation mechanism using a two-layer PDMS microfluidic device consisting of a flow layer and a control layer. The separation area is situated in the flow layer and consists of a 25 µm thick planar microchannel with a 12x128 matrix of funnel constrictions. In each row, the funnels have identical pore sizes (Figure 2C). In successive rows from the bottom to the top of the device, the funnel pore size is decreased from 12 µm to 2 µm (Figure 2B). Fluid flow in the device is controlled using on-chip microvalves V1-V6 (Figure 2A). When V1-V2 are open and V3-V6 are closed, a lateral flow is enabled to infuse sample cells into the funnel matrix. When V1-V2 are closed, the remaining valves act as a fluidic H-bridge to create the oscillatory flow used for separation.

Supporting microfluidic channels are added to generate precisely controlled pressure that is applied uniformly to each cell. To generate the uniform pressure, the oscillation pressure channels are bifurcated multiple times in order to generate distribu-
tion channels aligned to each column of constrictions with identical flow rate (Figure 2B). Fluid flow from these distribution channels recombine to form a uniform sheet flow in the matrix of funnel constrictions, which subjects each cell to the same viscous force from the fluid. Precise control of the fluid flow in the funnel matrix is established using elongated microchannels that add hydrodynamic resistance to the oscillation pressure inlets. These elongated channels present a dominant hydrodynamic resistance, enabling precise control of fluid flow using an external pressure controller.

The cell separation process begins by infusing the sample mixture into the bottom channel of the separation area. When V1-V2 are closed, then V3-V6 are used as described above to oscillate the sample vertically. Oscillation is applied with an upward bias such that cells travel through the device until they are retained by a sufficiently small pore. When steady state has been reached, V3-V6 are closed and V1-V2 are opened to extract the cells into outlets, while simultaneously bringing in new cells to restart the process.

**Figure 2:** Device concept schematic showing an overview of the layout including inlets, outlets and valves (A); the separation area with distribution channels above and below (B); and individual funnels (C)

**RESULTS AND DISCUSSION**

We characterized this device using L1210 mouse lymphoma cells (MLCs) and peripheral blood mononuclear cells (PBMCs). We initially sorted each cell type individually to obtain their characteristic distribution in the device, and then combined the two cell populations in order to demonstrate phenotypic separation. In the latter case, MLCs were stained with a fluorescent dye to distinguish them from PBMCs. For each experiment we injected a sample into the bottom row of the separation area and oscillated fluid flow in the device using a pressure of 7 kPa applied for 3 seconds in the forward direction followed by 1 second in the reverse direction. This process is repeated for a total of 60 seconds. The cells in each row are then counted manually using an optical microscope.

After sorting, both PBMCs and MLCs showed a narrow and consistent distribution in the funnel matrix. These distributions were not altered when a mixture of these cells was sorted in the device. Specifically, the peak of the PBMCs and MLCs were in the 6 and 9 µm rows respectively (Figure 3A). Dividing the outlet into two groups, where the ≥8 µm rows are used to collect
MLCs and ≤7 µm rows are used to collect PBMCs, would yield a separation efficiency of 98% with a purity of 99% for the MLCs and a separation efficiency of 97% with a purity of 95% for the PBMCs in this sample (Figure 3B).

![Figure 2: Distribution of PBMCs and MLCs after sorting of a mixed sample (A); separation efficiency and purity of sorted PBMCs and MLCs using a separation cutoff of 8 µm (B); and percentage of cells in a sample exhibiting ratcheting as a function of oscillation pressure (C).](image)

To investigate the effect of the oscillation pressure we sorted samples of MLCs at various pressures while using the same oscillation period as before. We observed that as the oscillation pressure increases, the mean of the cell distribution decreases toward smaller funnel rows. The spread of the distribution, characterized by the standard deviation from the mean, also increased with pressure. This shift is likely a result of cell deformability making a greater contribution than cell size to the steady-state distribution in the funnel matrix. Since more variability associated with cell deformability than with size, the sorted cells show a wider distribution at greater pressures. The tested cells were also stained with a simple live/dead stain. We observed that cell viability was preserved at all pressures used in testing.

To characterize the ratcheting effect we sorted samples at various oscillation pressures using 3 seconds of forward pressure followed by 1 second of reverse pressure. We oscillated for a total of 60 seconds and then immediately reversed the separation by applying an inverse oscillation of 3 seconds backward followed by 1 second forward over the next 60 seconds. The cells which were irreversibly separated remained caught in their sorted distribution whereas those that had not undergone ratcheting returned to the bottom row. The percentage of cells which exhibit ratcheting is shown in Figure 3C for oscillation pressures of 10, 14, 28 and 41 kPa. As the oscillation pressure is increased, a greater percentage of cells were observed to ratchet. When 41 kPa was used, the entire population is irreversibly separated. This irreversible separation process presents the potential for integration with downstream microfluidic processes.

ACKNOWLEDGEMENTS

This work is funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Michael Smith Foundation for Health Research (MSFHR). The authors would like to thank Elena Polishchuk, Dana Kyluik, and Mark Scott for providing materials used in this study.

REFERENCES


CONTACT

*H. Ma, tel: +1-604-827-4703; hongma@mech.ubc.ca*